

What is claimed is:

(96)

1. A method for mass production of dsRNA, which comprises:
  - a) providing nucleic acid target in a form replicable by an RNA-dependent RNA polymerase;
  - b) incorporating the nucleic acid target into the genome of an RNA virus or other RNA replicon encoding said polymerase, where said nucleic acid target is replicated by the polymerase encoded by the RNA virus or other RNA replicon under conditions sufficient for template-directed RNA synthesis in a living cell, one of the reaction products being necessarily double-stranded (ds) RNA; and
  - c) recovering said dsRNA products in a sufficiently pure form.
2. The method according to claim 1, wherein said nucleic acid target encodes a polypeptide or is equivalent to a noncoding region in the genome of a desired organism.
3. The method according to any one of the preceding claims, wherein the nucleic acid target is operably linked with determinants essential for detectable replication by the polymerase.
4. The method according to any one of the preceding claims, wherein the polymerase is a genetically modified or wild-type polymerase.
5. The method according to any one of the preceding claims, wherein the RNA virus or other RNA replicon is genetically modified or wild-type.
6. The method according to any one of the preceding claims, wherein the RNA replicon is RNA virus-like particle, viroid or RNA-based autonomous genetic element.
7. The method according to any one of the preceding claims, wherein the nucleic acid encoding the polymerase and the nucleic acid target are distinct nucleic acids.
8. The method according to any one of the preceding claims, wherein the RNA virus is an RNA bacteriophage.

9. The method according to claim 7 or 8, wherein the RNA virus is from a member of the *Cystoviridae* family, preferably from a bacteriophage selected from the group comprising  $\phi 6$ ,  $\phi 7$ ,  $\phi 8$ ,  $\phi 9$ ,  $\phi 10$ ,  $\phi 11$ ,  $\phi 12$ ,  $\phi 13$  and  $\phi 14$ , most preferably from bacteriophage  $\phi 6$ .

10. The method according to any one of the preceding claims, wherein the replicable form of the nucleic acid target is replicated in a prokaryotic cell, preferably in a gram-negative bacterial cell, more preferably in a bacterial cell selected from the group comprising *Pseudomonas sp.*, *Escherichia sp.* and *Salmonella sp.*, most preferably in a cell of *Pseudomonas syringae*.

11. The method according to any one of claims 1 to 10, wherein the replicable form of the nucleic acid target is replicated in a eukaryotic cell, such as mammalian, insect, plant or yeast cell.

12. The method according to any one of the preceding claims, wherein the nucleic acid target is delivered into the living cell using a suicide vector, preferably a DNA vector, most preferably a DNA plasmid.

13. The method according to any one of the preceding claims, wherein a suicide vector, comprising a target nucleic acid operably linked with sequences sufficient for detectable replication by the viral replication apparatus, is used to incorporate said nucleic acid target into the genome of said RNA virus.

14. A system for mass production of dsRNA, which comprises:

- a target nucleic acid sequence operably linked with determinants essential for replication by an RNA synthesis apparatus of an RNA virus or another RNA replicon;
- a living cell capable of supporting the replication of the RNA virus or other RNA replicon; and
- a recovery procedure for recovery of the dsRNA products in a sufficiently pure form.

15. The system according to claim 14, wherein the living cell is a carrier-state cell or can be transformed into carrier state.

16. The system according to claim 14 or 15, wherein the nucleic acid target is provided in a suicide vector.

17. The system according to any one of claims 14 to 16, wherein the RNA-dependent RNA polymerase in the RNA synthesis apparatus originates from a dsRNA virus or a dsRNA replicon.

18. The system according to any one of claims 14 to 17, wherein the RNA-dependent RNA polymerase in the RNA synthesis apparatus originates from the *Cystoviridae* family, preferably from a bacteriophage selected from the group comprising  $\phi 6$ ,  $\phi 7$ ,  $\phi 8$ ,  $\phi 9$ ,  $\phi 10$ ,  $\phi 11$ ,  $\phi 12$ ,  $\phi 13$ ,  $\phi 14$ , most preferably from bacteriophage  $\phi 6$ .

19. The system according to any one of claims 14 to 18, wherein the living cell is a prokaryotic cell, preferably a gram-negative bacterial cell, more preferably the bacterial cell is selected from the group comprising *Pseudomonas sp.*, *Escherichia sp.* and *Salmonella sp.*, most preferably the bacterium is *Pseudomonas syringae*.

20. A kit for mass production of dsRNA, wherein the kit comprises:

- a) a vector for transient expression of target nucleic acid in preselected cells that either are carrier-state or can be transformed into carrier state and/or
- b) a genetically modified virus into where the target nucleic acid can be introduced; and/or
- c) cells that either are carrier-state or can be transformed into carrier state.

21. A method for inducing sequence-specific gene silencing effects in eukaryotic systems, the method comprising:

- a) providing nucleic acid target in a form replicable by an RNA-dependent RNA polymerase;
- b) incorporating the nucleic acid target into the genome of an RNA virus or other RNA replicon, where said nucleic acid is replicated by the polymerase encoded by the RNA virus or other RNA replicon under conditions sufficient for template-directed RNA synthesis in a living cell, one of the reaction products being necessarily double-stranded (ds) RNA;

- c) recovering said dsRNA products in a sufficiently pure form and optionally modifying said products for optimal performance;
- d) using said pure, optionally modified, dsRNA products to induce sequence-specific gene-silencing effects in eukaryotic systems, such as organisms, cells or cell-free extracts.

22. The method according to claim 21, wherein the RNA-dependent RNA polymerase originates from a dsRNA virus or a dsRNA replicon.

23. The method according to claim 21 or 22, wherein the dsRNA virus is from the *Cystoviridae* family, preferably from a bacteriophage selected from the group comprising  $\phi 6$ ,  $\phi 7$ ,  $\phi 8$ ,  $\phi 9$ ,  $\phi 10$ ,  $\phi 11$ ,  $\phi 12$ ,  $\phi 13$ ,  $\phi 14$ , most preferably from bacteriophage  $\phi 6$ .

24. The method according to any one of claims 21 to 23, wherein the living cell is a prokaryotic cell, preferably a gram-negative bacterial cell, more preferably the bacterial cell is selected from the group comprising *Pseudomonas sp.*, *Escherichia sp.* and *Salmonella sp.*, most preferably the bacterium is *Pseudomonas syringae*.

25. The method according to any one of claims 21 to 24, wherein the optional step of modifying for optional performance is fragmenting dsRNA with dsRNA-specific ribonucleases, preferably RNase III, Dicer, or derivatives thereof.

26. The method according to any one of claims 21 to 25, wherein the target nucleic acid is provided in a suicide vector.

27. The method according to any one of claims 21 to 26, wherein the dsRNA products are used to induce sequence-specific gene-silencing effects in invertebrate animal systems, preferably of insect or nematode origin, most preferably from *Drosophila melanogaster* or *Caenorhabditis elegans* origin.

28. The method according to any one of claims 21 to 27, wherein the dsRNA products are used to induce sequence-specific gene-silencing effects in vertebrate animal systems, preferably of mammalian origin, most preferably of human or mouse origin.

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